

Amendments to the Claims

The following listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

What is claimed is:

1. (Currently amended) A nucleic acid comprising a sequence coding for a fusion protein, the sequence comprising:



where

~~F is a nucleic acid sequence coding for an amino acid sequence which allows secretion of a protein encoded by Y into a fermentation medium~~ comprises a nucleic acid sequence encoding a hirudin derivative,

~~As is a chemical bond or a nucleic acid sequence comprising a codon,~~

~~m is an integer from 0-10,~~

~~R is a chemical bond or an arginine codon,~~

~~n is 0 or 1, and~~

~~Y is a nucleic acid sequence coding for a protein of interest.~~

2. (Currently amended) The nucleic acid of claim 1, wherein the nucleic acid comprises:



where

P is a promoter,

S is a nucleic acid sequence coding for a signal sequence which increases yield, and

~~T is an untranslated expression enhancing nucleic acid sequence,~~

wherein F, As_m, R_n, and Y are as defined in claim 1.

3. (Original) The nucleic acid of claim 2, wherein S is the oprF gene from *Pseudomonas fluorescens*, the nucleic acid encoding the signal sequence of

Salmonella typhimurium outer membrane protein (fim D), the nucleic acid sequence encoding the signal sequence of the *Escherichia coli* alkaline phosphatase precursor protein, the nucleic acid sequence encoding the signal sequence smompA derived from the ompA gene for major outer membrane protein of *Serratia marcescens*, the nucleic acid sequence encoding the signal sequence ecoompC derived from *Escherichia coli* ompC gene coding for major outer membrane protein, the nucleic acid sequence encoding the signal sequence af009352 derived from *Bacillus subtilis* osmoprotectant binding protein precursor (opuCC), the nucleic acid sequence encoding the signal sequence aeoxyna derived from *Aeromonas caviae* xynA gene for xylanase I precursor, or the nucleic acid sequence encoding the signal sequence stomps1 derived from *Salmonella typhi* gene for outer membrane protein S1.

4. (Currently amended) The nucleic acid of claim 2, wherein the nucleic acid sequence F encodes for lepirudin, Val-Val-hirudin, Ile-Thr-hirudin, Ser-hirudin or Ala-hirudin.

5. (Original) The nucleic acid of claim 2, wherein the protein of interest comprises proinsulin, insulin, or derivative thereof.

6. (Canceled).

7. (Withdrawn) A protein encoded by the nucleic acid of claim 1.

8. (Withdrawn) The protein of claim 7, wherein the protein of interest is correctly folded as part of the fusion protein in the fermentation medium.

9. (Original) A plasmid comprising the nucleic acid of claim 1.

10. (Original) A host cell comprising the plasmid of claim 9.

11. (Original) A host cell comprising the nucleic acid of claim 1.

12. (Original) The host cell of claim 10, wherein the host cell is selected from *Escherichia coli*, *Bacillus subtilis*, and *Streptomyces lividans*.

13. (Original) The host cell of claim 11, wherein the host cell is selected from *Escherichia coli*, *Bacillus subtilis*, and *Streptomyces lividans*, and wherein the nucleic acid is optionally integrated in the genome of the host cell.

14. (Currently amended) A process for fermentative production of a fusion protein, comprising: ~~expressing the nucleic acid of~~ fermenting the host cell of claim 11 in a fermentation medium resulting in a fermentation supernatant ~~to form the fusion protein;~~ and isolating the fusion protein produced thereby.

15. (Canceled).

16. (Currently amended) The process of claim 14, wherein isolating the fusion protein comprises precipitating the fusion protein from ~~the supernatant containing the fusion protein~~ [[a]] the supernatant and concentrating the fusion protein by one of microfiltration, hydrophobic interaction chromatography, and ion exchange chromatography.

17. (Currently amended) The process of claim 14, wherein isolating the fusion protein comprises precipitating components of a culture medium or the supernatant ~~containing the fusion protein~~, while the fusion protein remains in solution.

18. (Currently amended) The process of claim 14, ~~wherein expressing the nucleic acid in the host cell comprises fermentation resulting in a fermentation supernatant,~~ and wherein after the fermentation, mercaptan or cysteine hydrochloride is added to the fermentation supernatant at pH about 6 to 9, resulting in a free SH group concentration of about 0.05 to 2.5 mM.

19. (Currently amended) The process of claim 14, wherein: ~~expressing the nucleic acid in the host cell comprises fermentation resulting in a fermentation supernatant,~~ isolating the fusion protein comprises separating the fermentation supernatant from the host cell, and after separating the fermentation supernatant from the host cell, the host cell is repeatedly cultured in fresh medium to form additional supernatant from each culture, and fusion protein is isolated from each additional supernatant.

20. (Currently amended) The process of claim 14, wherein: ~~expressing the nucleic acid in the host cell comprises forming a supernatant containing the fusion protein,~~ and mercaptan or ~~cystein~~ cysteine hydrochloride is added to the supernatant at pH about 6 to 9, so that the supernatant has a free SH group concentration of about 0.05 to 2.5 mM.

21. (Currently amended) A process for the production of insulin or an insulin derivative, comprising:

obtaining fusion protein by fermenting a host cell comprising the nucleic acid of claim 5 in a fermentation medium and isolating the fusion protein produced thereby,

releasing insulin or insulin derivative from the fusion protein by enzymatic or chemical cleavage; and

isolating the insulin or insulin derivative.

22. (Canceled).

23. (Original) The process of claim 14, wherein the host cell comprises a bacterium.

24. (New) A nucleic acid comprising a sequence coding for a fusion protein, the sequence comprising:

—F—As_m—R_n—Y—

where

F comprises a nucleic acid sequence encoding hirudin or a hirudin derivative,

As is a nucleic acid sequence comprising a codon,

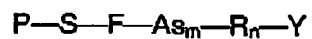
m is an integer from 0-10,

R is an arginine codon,

n is 0 or 1, and

Y is a nucleic acid sequence coding for proinsulin, insulin, or a derivative thereof.

25. (New) The nucleic acid of claim 24, wherein the nucleic acid comprises:



where

P is a promoter,

S is a nucleic acid sequence coding for a signal sequence which increases yield, and

wherein F, As_m , R_n , and Y are as defined in claim 24.

The Applicants respectfully submit that the claims, as amended, are in condition for allowance, and respectfully request early, favorable action on the application. Should the Examiner believe that an interview would advance the prosecution of this application, the Applicants invite her to contact the undersigned at 908.231.3444.

Respectfully submitted,



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